## Figure 1A

**Control**

0  5  10  20  40  60

**Carbachol**

0  5  10  20  40  60 sec

MYPT1 -



## Figure 1B

**Control**    **Carbachol**

# Figure 2A



# Figure 2B



# Figure 2

**Figure 3B**

210 →
97 →
67 →
45 →
31 →

**Figure 3D**

45 kDa -
31 kDa -

**Figure 3A**

NaCl (mM)

SMPP-1M Kinase Activity (pmol/min)

Fraction

**Figure 3C**

cpm released

15000
10000
5000
0

S
T
Y

K K K R Q S R R S T Q G V T L
Cycle
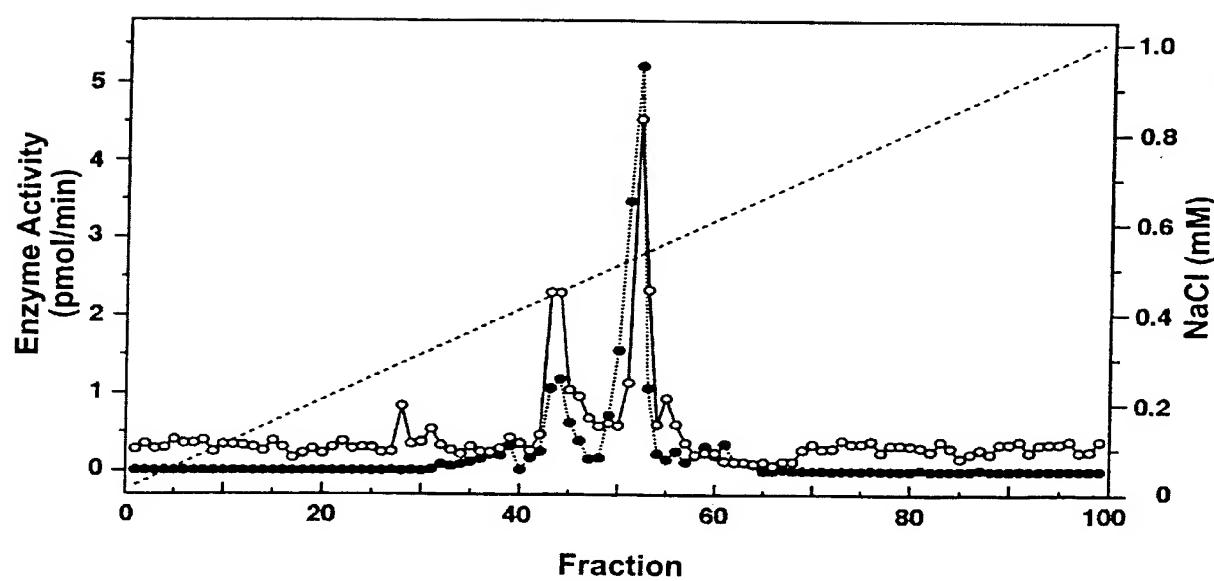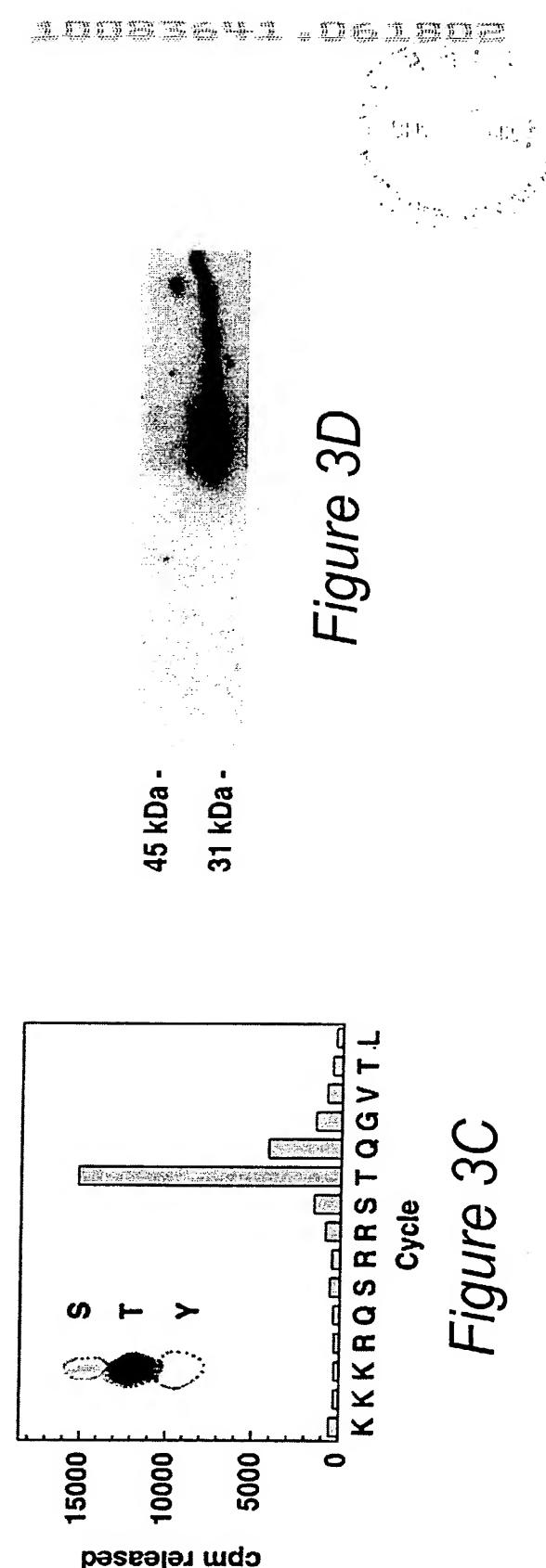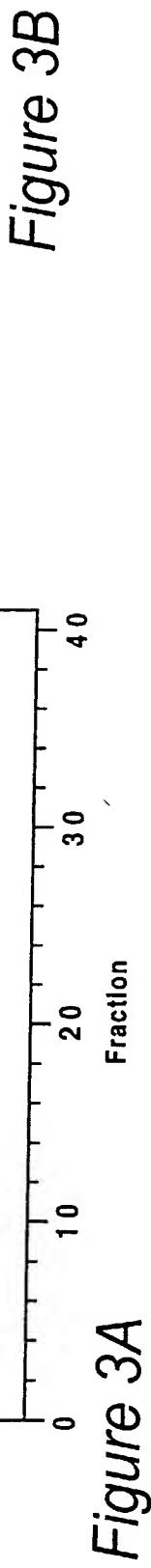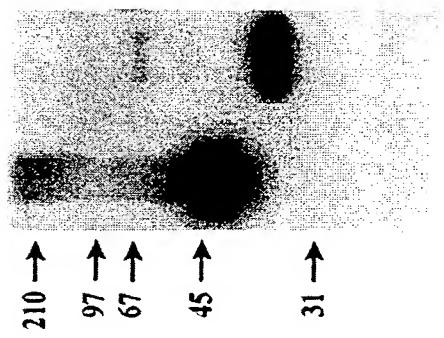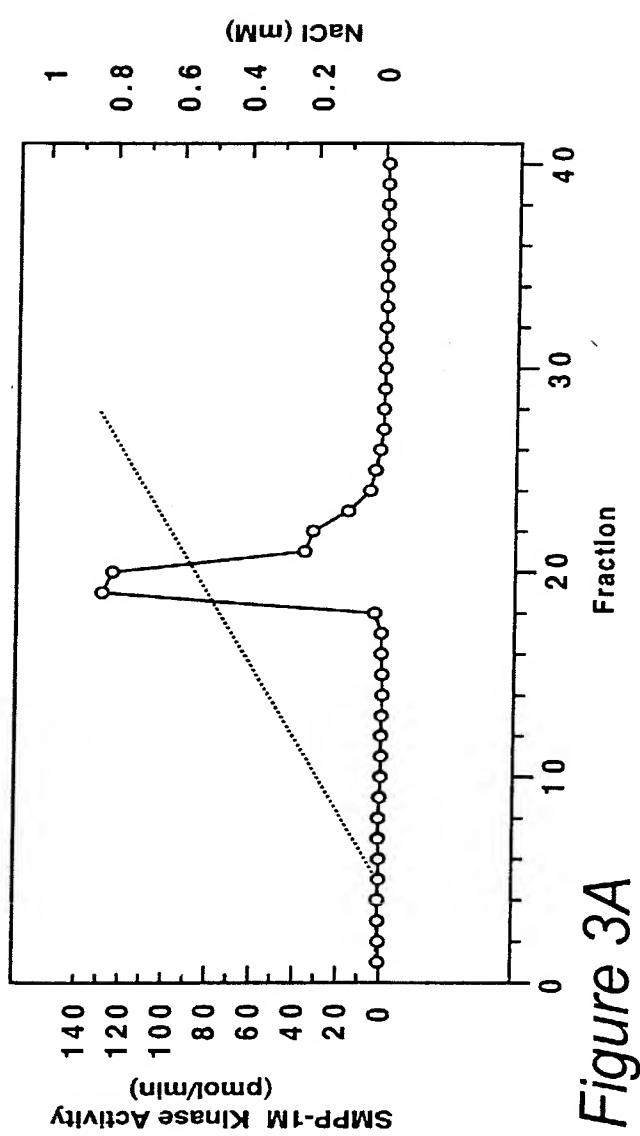
| Edman cycle number | Amino acids recovered at each cycle | | | FASTF Aligned Sequence | Called Protein |
|---|---|---|---|---|---|
| | M | M | M | MGEELGSGQFAIV---------- | |
| 1. | D | G | T | L | ::::::::::::: | |
| 2. | E | I | L | V | MSTFRQEDVEDHYEMGEELGSGQFAIVRKCRQKGTGKEYAAKFIKKRRLPSSRRGVSREEIEREVNILREIRHPNIITLH | |
| 3. | A | T | D | E |     10    20    30    40    50    60    70    80 | |
| 4. | Q | K | L | X | | |
| 5. | N | R | G | X | ----------------------------------------MLLDKXIFXRPIQ-- | |
| 6. | I | S | L | X |                    ::::: . . ; | |
| 7. | Y | F | G | X | DIFENKTDVVLILELVSGGELFDFLAEKESLTEDEATQFLKQILDGVHYLHSKRIAHFDLKPENIMLLDKNVPNPRIKLI | ZIP kinase |
| 8. | Q | Y | L | X |     90   100   110   120   130   140   150   160 | |
| 9. | R | K | F | X | | |
| 10. | A | P | T | X | ----------------------------------------------------------- | |
| 11. | I | T | G | I | DFGIAHKIEAGNEFKNIFGTPEFVAPEIVNYEPLGLEADMWSIGVITYILLSGASPFLGETKQETLTNISAVNYDFDEEY | |
| 12. | V | E | Q | X |    170   180   190   200   210   220   230   240 | |
| | | | | ---------------MTIAQNLXYXXXIX----- | |
| | | | |                 :::::: . . : | |
| | | | | FSSTSELAKDFIRRLLVKDPKRRMTIAQSLEHSWIKVRRREDGARKPERRRLRAARLREYSLKSHSSMPRNTSYASFERF | |
| | | | |    250   260   270   280   290   300   310   320 | |

*Figure 4*

## Figure 5B



Enzyme Reaction Rate (pmol/min)

[Peptide] μM

## Figure 5A



Relative SMPP-1M Kinase Activity

Y27632 (μM)

## Figure 5D

Phospho-aa analysis

$^{32}P$ release from Edman sequencing

E S L T E D A X Q



## Figure 5C

SMPP-1M kinase | Rho kinase

C130 514-963 | rM133 full length | C130 514-963 | rM133 full length | MW



— 200

— 116

— 98

— 67

— 45
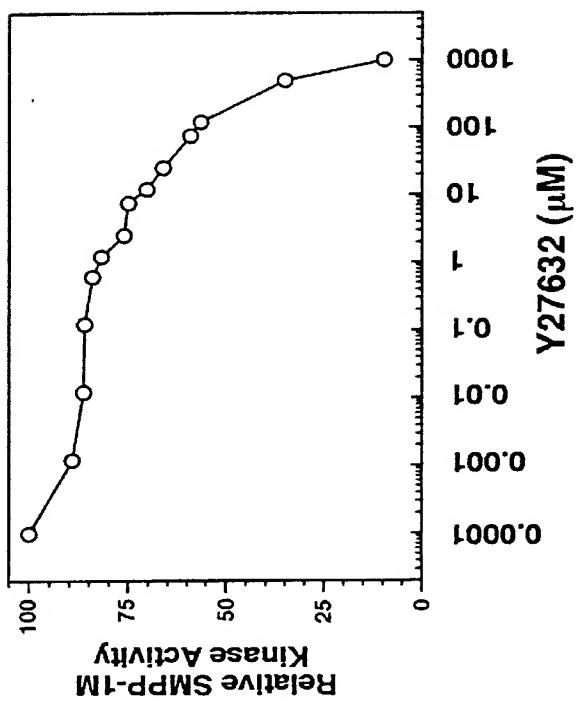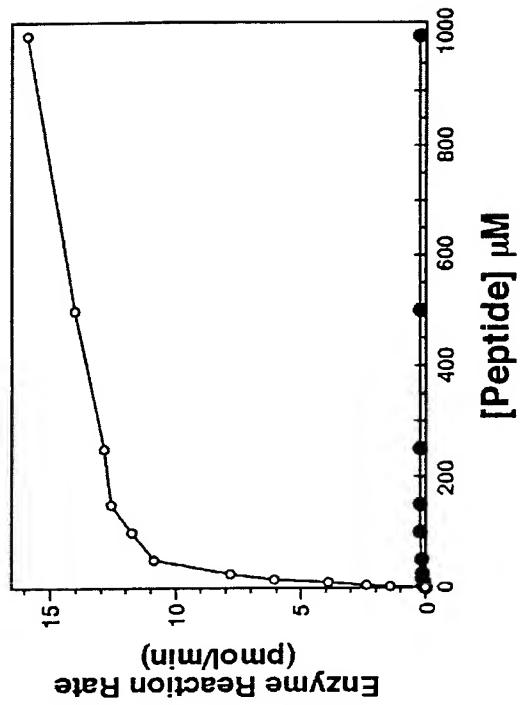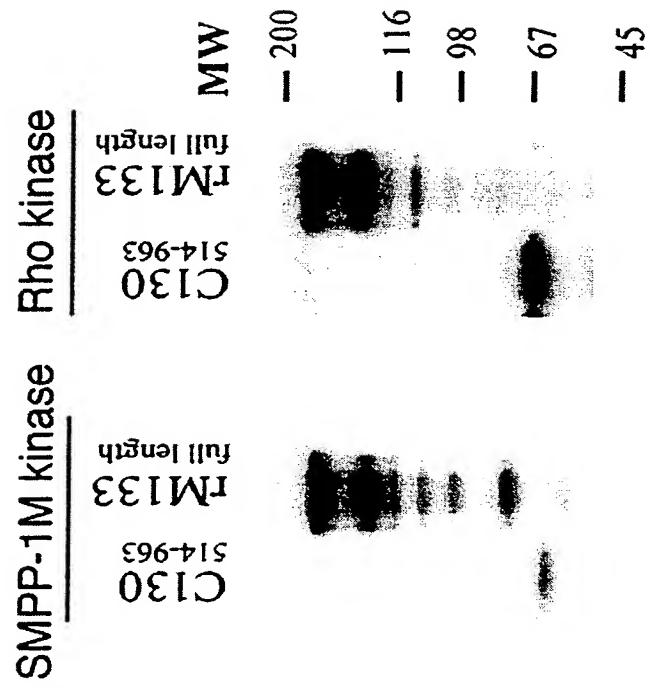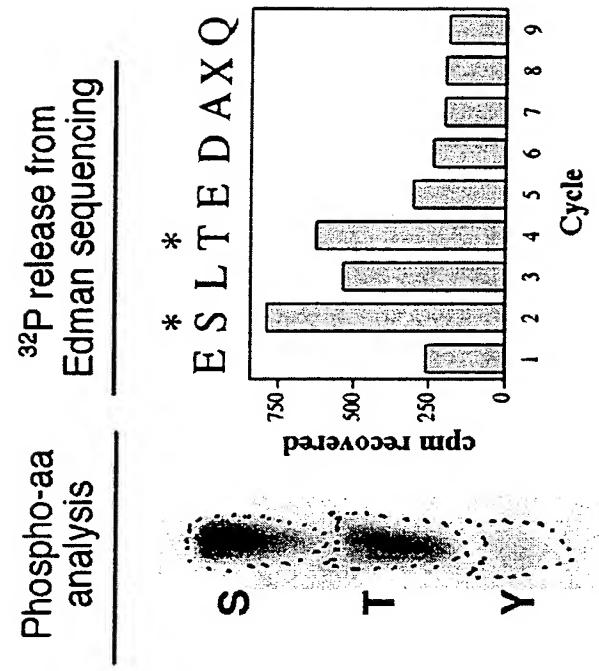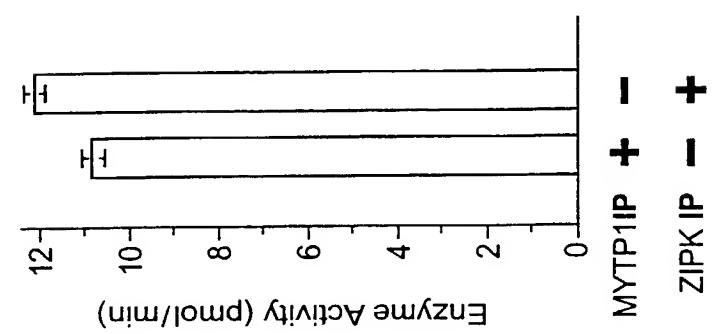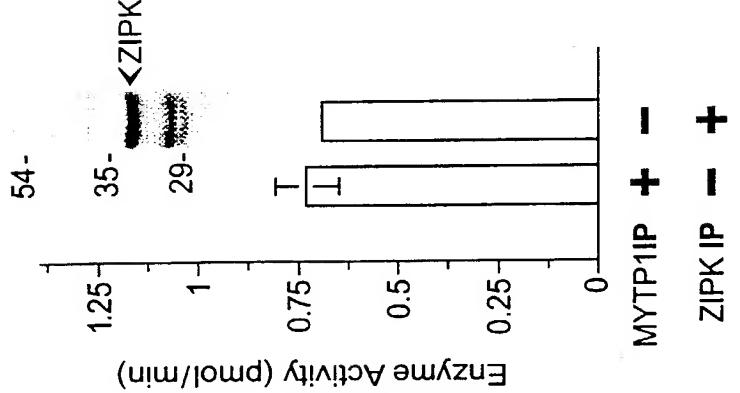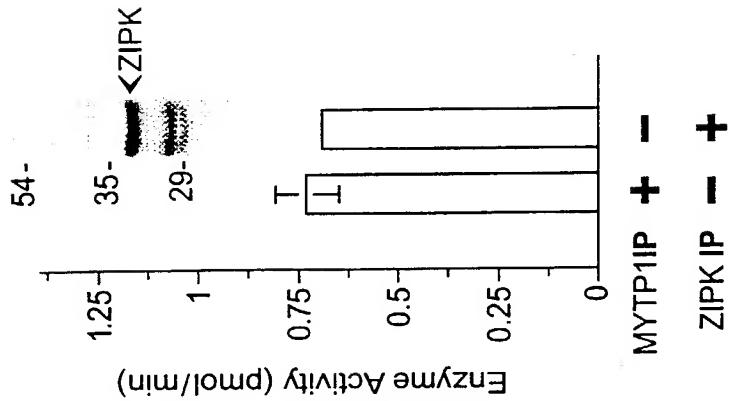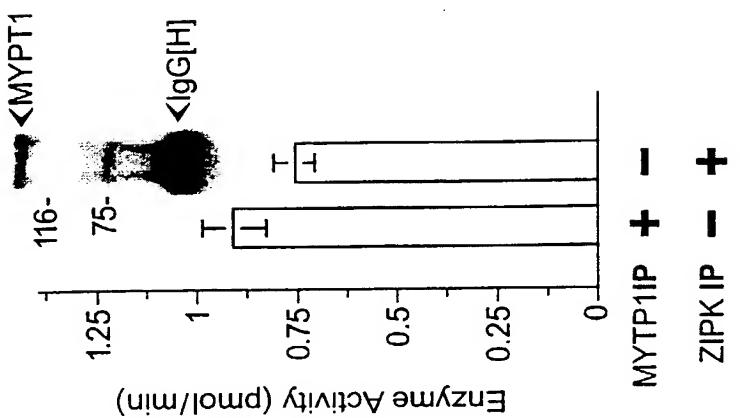
## Figure 6E



## Figure 6C

## Figure 6D
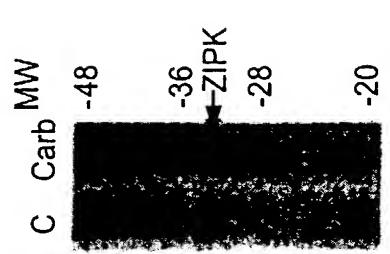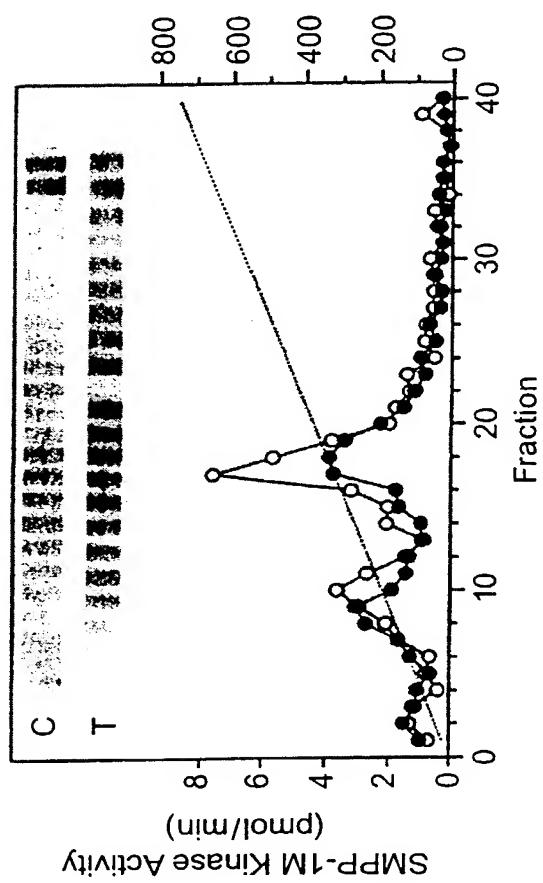


## Figure 6B

## Figure 6A

*Figure 7A*

*Figure 7B*

*Figure 7C*

# Figure 8

Putative nucleotide sequence of smooth muscle MYPT-kinase showing start site in bold.

GNTATGNATA TCGGTTTAAT CGGCCGGAGC TCGCCCNCNG GGCAGCTGGA

| | | | | | |
|---|---|---|---|---|---|
| CTCCCTCTCA | GACCTCCTTC | TTTCTCGCCC | TCAGCACGGG | ATTAACCTCA | 100 |
| CTTGACTGTT | CTTGGGTCCC | CGGTGCCGGG | CCAGCGTCCT | CTCCCTCAAG | 150 |
| GCAATCCCCA | AGTGTCTGTC | ATGAGGCTCT | TTGGGCAGTT | CTGTTGTTGT | 200 |
| GGGAAACCTG | GGAACAGATG | CACAGAGGCT | GGGGTACAGA | GTCCTGCCTT | 250 |
| CCTCTGGGTC | TGCAGCGCTT | AGCTGTTCCT | TCCCCCACAG | CGGCCAGTTC | 300 |
| GCCATCGTGC | GCAAGTGCCA | GCAGAAGGGC | ACCGGCATGG | AGTACGCGGC | 350 |
| CAAGTTCATA | AAGAAGCGGC | GCCTGCCGTC | CAGCCGGCGC | GGTGTGAGCC | 400 |
| GTGAGGAGAT | CGAGCGCGAG | GTGAGCATCC | TGCGCGAGAT | CCGCCACCCC | 450 |
| AACATCATCA | CGCTGCACGA | TGTGTTCGAG | AACAAGACAG | ATGTGGTGCT | 500 |
| GATCTTGGAG | CTGGTGTCCG | GCGGCGAACT | TTTCGACTTT | CTGGCTGAGA | 550 |
| AGGATCACTG | ACAGAGGATG | AGGCCACGCA | GTTCCTCAAG | CAGATCCTGG | 600 |
| ACGGTGTCCA | CTACCTGCAC | TCCAAGCGCA | TCGCGCACTT | TGACCTGAAG | 650 |
| CCGGAGAACA | TCATGTTGCT | GGACAAGCAT | GCAGCCAGCC | CACGCATTAA | 700 |
| GCTCATCGAC | TTTGGCATCG | CGCACAGGAT | CGAGGCCGGT | AGCGAGTTCA | 750 |
| AGAACATCTT | TGGCACGCCA | GAGTTCGTCG | GTGAGGGGCA | GGTGTGGGCA | 800 |
| CCACCCGATA | GGGTAGATTT | TGCACGGCCT | TGGCCTGACC | TGCCTCAACA | 850 |
| ATCCTGTCTT | CCACAGCCCC | TGAGATTGTA | AACTATGAAC | CACTTGGCTT | 900 |
| GGAAGCTGAT | ATGTGGAGCA | TCGGCGTCAT | CACCTACATC | CTGTGAGTGC | 950 |
| CTGAGATGGG | CAGGGGCCTC | AGACTGTACC | TGCTAGAGGC | CCAGGGATCA | 1000 |
| GGGCTGGCAC | CTCTGCAAAC | TGCAAACACT | GGGGCTGAGA | GATGTCCCTG | 1050 |
| GGAACNCTGG | ATATGCCTGG | GCCCCACCAA | NGTAGGACCA | TNC | 1093 |

# Figure 9

Deduced amino acid sequence of rat aorta smooth muscle MYPT-kinase (underlined shows alignment with 52kDa ZIP kinase sequence)

XMXIGLIGRS SPXGQLDSLS DLLLSRPQHG INLT*LFLGP RCRASVLSLK
AIPKCLS*GS LGSSVVVGNL GTDAQRLGYR VLPSSGSAAL SCSFPH<u>SGOF</u>
<u>AIVRKCOOKG TGMEYAAKFI KKRRLPSSRR GVSREEIERE VSILREIRHP</u>
<u>NIITLHDVFE NKTDVVLILE LVSGGELFDF</u> LAEKDH*QRM RPRSSSSRSW
TVSTTCTPSA SRTLT*SRRT SCCWTSMQPA HALSSSTLAS RTGSRPVASS
RTSLARQSSS VRGRCGHHPI G*ILHGLGLT CLNNPVFHSP *DCKL*TTWL
GS*YVEHRRH HLHPVSA*DG QGPQTVPARG PGIRAGTSAN CKHWG*EMSL
GTLDMPGPHQ XRTX